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ON THE DEVELOPMENT OF THE SPONTANEOUSLY PARTHENOGENETIC EGGS OF ASTERINA (PATIRIA) MINIATA.

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INTRODUCTION.

While engaged in a series of experiments on echinoderm hybridology, which I was conducting during the months of April and May, 1920, at Pacific Grove, California, I was forcibly struck by the frequency with which spontaneous parthenogenesis occurs in the starfish, *Asterina* (*Patiria*) *minata*. When I use the term "spontaneous," I mean that eggs were in no way treated either by physical or by chemical agents. Precautions were taken, moreover, to prevent accidental fertilization. The procedure was as follows:

Sea water, brought in from the open sea and therefore free from the chemical impurities present in sea water that has been pumped through metal pipes, was allowed to stand at least four days at laboratory temperatures, which during the month of May ranged from 16° to 19° C. It is certain that no sperms could live for this length of time in sea water. This method is chosen in preference to Loeb's practise of heating the sea water to 60° C. for some time, because it involves no possible chemical changes in the sea water nor any driving out of oxygen. Before opening a starfish, it was scrubbed thoroughly in cold running fresh water and rinsed in a strong stream of fresh water. In case the animal proved to be a male it was discarded, and hands and instruments were scrubbed in fresh water before touching another starfish. If the animal proved to be a ripe female the ovaries were gently shaken into a finger-bowl containing 150 c.c. of the sea water prepared for the purpose and the bowl was covered with a clean glass plate and placed upon a table out of reach

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of direct sunlight. For purposes of observation eggs were removed from time to time and placed in watch-glasses with a sterilized pipette. It was thought safer not to run, as direct controls, normally fertilized eggs, but on other days and under identical conditions numerous observations were made on the course of normal fertilization and development. The differences observed between the behavior of parthenogenetic and that of fertilized eggs were so striking that it seemed well worth while to study and to describe them.

Peculiarities of the Material Used.

Asterina (*Patiria*) *miniata* is one of the commonest starfishes of the California coast. It is a relatively small species in which the five rays are almost completely amalgamated with the central disc in such a way as to give the creature a nearly pentagonal outline. In color it ranges from a brilliant scarlet to a light cream color with various intergrades and piebald combinations. It seems likely that there are several subspecies that freely interbreed. My experience seems to indicate that relatively few eggs mature at a time and are exuded in small numbers over a long season. Among the very large number of females examined I never found an ovary that showed any large percentage of ripe eggs. Those that were shed from the removed ovary and gently shaken in sea water contained oöcytes in all stages of development, some quite small, others fully grown but incapable of maturation, still others mature and ready for maturation and fertilization after standing about one and a half to two hours in sea water. None of the eggs when freshly shed had undergone maturation. This may mean that the artificial shedding of the eggs is a premature process, and that, as seems to be the case in some asteroids, if the eggs were to be normally extruded through the genital pores, they would be immediately ready for fertilization. I have never been able to observe the extrusion of eggs in *Asterina,* nor to induce it by massaging, as can be done in some asteroids. This may possibly be due to some peculiar sexual rhythm in this species, which results in ovulation occurring only at night or at some particular phase of the moon or of the tide.

On this point I have no evidence. So it should be borne in mind that the eggs used in these experiments, and presumably by Loeb, who did a considerable amount of work upon artificial parthenogenesis in this species, are not the normally shed eggs equivalent to those which are fertilized in nature, but are probably prematurely shed eggs. It is very questionable, therefore, whether parthenogenesis ever occurs in nature. It seems more probable that the parthenogenetic eggs observed in these experiments result from the artificial conditions involved in shedding some of the eggs prematurely.

The eggs of *Asterina* are very hardy and resistant of the cytolytic action of sea water. While the unmaturated oöcytes of most echinoderms begin to disintegrate within twenty-four hours, those of *Asterina* frequently remain unchanged, as though in stable equilibrium, for from four to eight days. I have before me a number of microscopic whole mounts, showing numbers of eggs and larvæ of *Asterina* fixed in Bouin's solution on the eighth day after fertilization, in which there occur a number of unmatured oöcytes with germinal vesicle clean-cut and spherical and plasmosome well defined. This ability of the unripe eggs to withstand disintegration is of great practical value in the study of development, for the water is kept free from the products of egg decay; a decided advantage in view of the fact that where there are small percentages of developing eggs surrounded by large percentages of non-developing eggs, the former would have very small chance of survival if the latter were to decay and foul the water.

Experimental Data.

Most of the detailed data on pathenogenesis in *Asterina* were obtained during the month of May, 1920, although the phenomenon was noted incidentally throughout April. The month of May seems to be the best month for work with *Asterina* as there appear to be larger ovaries and more full-grown oöcytes then than earlier. Possibly June would be still better, though I have not tried any experiments at that time.

Some thirty-two experiments were made in all, and no two gave exactly identical results. A large series of ten experiments

made on May 20 shows the full range of diversity and will serve to illustrate all of the points of interest. As the time element is of prime importance in this study it is necessary to give readers as a norm for purposes of comparison a time schedule of the development of the normally fertilized *Asterina*.

TIME SCHEDULE OF THE DEVELOPMENT OF NORMALLY FERTILIZED EGGS OF ASTERINA.

Hours after Shedding.	Condition of Maturated and Fertilized Eggs and Embryos.
2¼	Fertilization membranes formed on majority of matured eggs.
3½	Cleavage beginning.
5	Cleavage taking place in all fertilized eggs, a few 4 and 8 cell stages.
7	Many stages as advanced at 32 + cells.
9	Most of eggs in early blastula stages, but there are a good many eggs without membranes, in early cleavage stages. These are probably parthenogenetic.
25	Two distinct types of larvæ present: the great majority being typical gastrulæ, swimming up near the surface of the dish, and capable of being readily pipetted off; a small minority of living larvæ variously abnormal and swimming at or near the bottom. These latter are probably, though not certainly, parthenogenetic.
49	Normal larvæ, early bipennariæ, forming enterocoel pouches.
73	Bipennariæ with well-defined ciliated bands.
96	Advanced bipennariæ with mouth, œsophagus, stomach, intestine, anterior and posterior enterocœls, waterpore, etc.

TIME SCHEDULE OF THE DEVELOPMENT OF PARTHENOGENETIC EGGS OF ASTERINA.

No. of Experiment.	Hours after Shedding.	Condition of Maturated Eggs or of Developing Embryos.
I	7	No membranes and no cleavage.
	9	About 1 per cent. of eggs show distinct membranes, no cleavage.
	24	About ½ of 1 per cent. swimming blastulæ, all subnormal in appearance, irregular in shape or solid. About 2 per cent. early cleavage stages (2 and 4 cell stages).
	48	All larvæ and cleavage stages undergoing cytolysis.
II	7	No membranes and no cleavage.
	9	No membranes and no cleavage.
	24	No larvæ nor cleavage.
III	6½	No membranes and no cleavage.
	8¾	No membranes, but about 1½ per cent. of cleavage stages, mostly fairly regular 2 and 4 cell stages.
	25	Nearly 1 per cent. blastulæ, mostly solid and motionless.

IV..... 6.....No membranes and no cleavage.
8½.....About 2 per cent. of eggs cleaving without membrane formation, some as advanced as 8 cells.
25.....Nearly 2 per cent. blastulæ, slightly abnormal and motionless.

V..... 6½.....About 5 per cent. of eggs with rather narrow but distinct membranes, no cleavage.
7½.....About 1½ per cent. of eggs without membranes in early cleavage stages (2, 3, 4 cells), some quite regular, but the majority more or less irregular.
8½.....About 5 per cent. of eggs cleaving, ranging from 2 to 16 cells. Evidently a good deal of cleavage has begun since the 7½ hour observation. A few of the eggs now show wide typical membranes and there are transitional stages between these and the more plentiful type with narrow membranes.
26.....About 7 per cent. swimming blastulæ, some nearly normal, but the majority solid, wrinkled or otherwise abnormal. Eggs with membranes, undergoing black cytolysis.
31.....Many gastrulæ, some with two or more archentera some exogastrulæ, etc.
74.....A good collection of twin larvæ, studied in a subsequent connection. No normal larvæ. All larvæ swimming on the bottom of the dish.

VI..... 6¾.....About 4 per cent. of eggs with distinct but narrow membranes, no cleavage.
8¼.....About 2½ per cent. of eggs with wide, typical membranes, but no egg with membrane shows cleavage; about ½ of 1 per cent. of eggs showing cleavage stages, ranging from 2 to 8 cells, some quite regular.
26½.....A very few larvæ, all subnormal, some motionless, others feebly swimming.

VII..... 7.....No membranes, no cleavage.
9.....No membranes, but about 2 per cent. of eggs in cleavage stages ranging from 2 to 32 cells.
26½.....About 1 per cent. subnormal blastulæ, a few swimming.
32.....A very few larvæ undergoing gastrulation; several with two or more archentera.

VIII..... 6½.....No membranes, no cleavage.
9.....No membranes, but about 3½ per cent. of eggs in cleavage stages, ranging from 2 to 16 cells.
26.....About 2½ per cent. of larvæ, all subnormal.

IX..... 7.....About 8 per cent. of maturated eggs with narrow membranes; about 3 per cent. of eggs without membranes showing first steps in cleavage, a few having completed the first cleavage.

8½.....A few eggs in 4- and 8-cell stages.

30½.....A fraction of 1 per cent. of larvæ undergoing gastrulation, and swimming about, the best of them being nearly normal in appearance.

78.....All larvæ dead.

X..... 7¼.....About 5 per cent. of eggs with fully typical membranes, no cleavage stages.

9½.....Over 50 per cent. of maturated eggs in cleavage stages, without membranes, ranging from 2 to 8 cells. No eggs with membranes segmenting.

31.....About 75 per cent. of all maturated eggs have undergone cleavage without membrane formation, and are in various stages ranging from early cleavage to gastrulæ. Numerous dwarf blastulæ, due to blastolomy; many gastrulæ with plural archentera; solid blastulæ and exogastrulæ in considerable numbers. All were swimming about on the bottom of the bowl. This culture was made the basis of a study of the more advanced development of parthenogenetic eggs and will be referred to in more detail in a subsequent discussion.

Significant Points brought out by the Data Shown in the Above Schedule.

1. Membrane formation occurs in exactly half of the experiments here described. It was observed in from 1 to 8 per cent. of the maturated eggs.

2. The degree of completeness of membrane formation varies greatly in different sets of eggs and in different eggs of a given set. In some eggs the membrane is so little lifted from the surface of the egg as to be scarcely noticeable, but in others the membrane is indistinguishable from that seen in fertilized eggs.

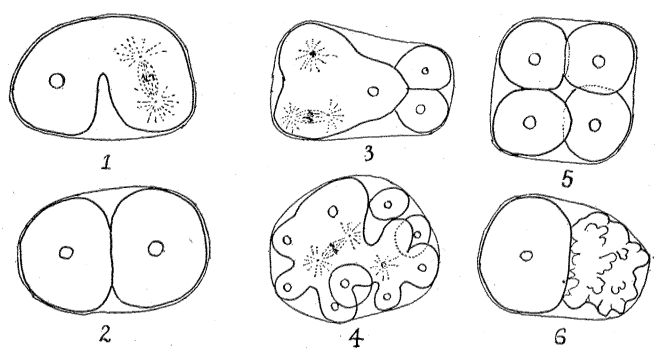
3. Eggs that form membranes, whether narrow or wide, do not further develop, but undergo cytolysis within twenty-four hours.

4. The percentage of maturated eggs that undergo parthenogenetic development varies from none to about seventy-five; in experiment II no cleavage occurred, while in experiment X 75 per cent. of all maturated eggs at least began cleavage. The aver-

age number of parthenogenetic eggs is about two per cent, of maturated eggs.

5. Eggs that undergo parthenogenetic cleavage never form "fertilization" membranes, the closely fitting vitelline membrane being the only envelope that surrounds the blastomeres.

6. Cleavage in parthenogenetic eggs never begins earlier than six and one half hours after the eggs are placed in sea water and the average time for the beginning of cleavage is about seven and a quarter hours. Cleavage begins in fertilized eggs sometimes as early as three and one half hours, and the average time of beginning is about four hours. Subtracting two hours for maturation to complete itself, we have cleavage beginning five hours after maturation in parthenogenetic eggs and two hours after maturation in fertilized eggs. There is, therefore, a retardation in development in the case of parthenogenetic eggs of three hours, and at a very critical period.



FIGS. 1–6. Cleavage stages in spontaneously parthenogenetic eggs of *Asterina*. *1*. An incompletely segmented two-cell stage in which one blastomere is in advance of the other. *2*. A normal two-cell stage. *3* and *4*. Irregular cleavage stages. *5*. A typical normal four-cell stage. *6*. A rather common type of abnormal cleavage in which one blastomere is undergoing cytolysis and the other is remaining normal.

7. Cleavage and subsequent development in parthenogenetic eggs take place much more slowly than in fertilized eggs. Even the most nearly normal parthenogenetic eggs take nearly twice as long to reach a given stage as do fertilized eggs. Development is, therefore, greatly retarded and we would naturally ex-

pect the larvæ to exhibit the various types of developmental defects that are commonly seen in inhibited individuals.

8. In none of the numerous experiments did parthenogenetic eggs give rise to even approximately normal bipennariæ. The most successful larvæ were certain double monsters that will be discussed later.

9. The average viability of parthenogenetic larvæ varies greatly in different sets. As a rule viability was lowest in those sets in which the smallest percentage of larvæ occurred and highest in those in which the largest percentage of larvæ occurred.

10. Individual viability varies greatly within a given set of eggs. Quite frequently eggs die and disintegrate during the first or subsequent cleavages, while it was not uncommon for a few larvæ in each of the best sets to live for from four to seven days.

11. Cleavage in parthenogenetic eggs is sometimes very normal in appearance, but in every set the majority of cleavage stages are irregular (Figs. 1–6). Sometimes blastomeres of the two cell stage separate, and form half-sized blastulæ, seldom going further. In other cases one or more blastomeres cease cleavage while the rest go on and form a covering of small cells about a large central cell. Numerous other cleavage anomalies occur which need not be detailed here.

Discussion.

Loeb's Observations of Spontaneous Parthenogenesis in Asterina.

Doubtless the reader recalls the work of Loeb (1905) on "Artificial membrane formation and chemical fertilization in a starfish (Asterina)." In this paper the author describes various methods employed first, for inducing membrane formation and second, for inducing cleavage and subsequent development in the same species of starfish which forms the material of the present investigation. Loeb recognizes the occurrence of spontaneous parthenogenesis in *Asterina* as is shown by the following quotations: "The eggs of the starfish show a slight tendency to develop spontaneously without any external influence." "If the eggs of *Asterina* are allowed to mature in sea water and are left to themselves, sometimes none, sometimes a fraction of a per cent., some-

times more, will segment and develop into larvæ. But the development of these eggs is much slower than that of fertilized eggs and, as a rule the larvæ are not so perfect and die sooner." "We have, therefore, two types of development in these (*Asterina*) eggs. One type is represented by the fertilized egg, and this type can be produced artificially in a number of eggs, at least, by calling forth the membrane formation by the above-named artificial means. The second type is represented by the spontaneously developing egg in which no membrane has been called forth; these latter eggs begin to segment later, and possibly develop more slowly than the other eggs, and form larvæ which are not as perfect as those belonging to the first type."

It will be seen that Loeb has touched upon some of the essential points that are brought out in my experiments. He notes that spontaneous parthenogenesis occurs in a small per cent. of eggs; that parthenogenetic cleavage takes place without membrane formation; that cleavage begins later; and that development is slower and less normal than is fertilized eggs. Loeb, however, was not primarily interested in the course or results of spontaneous parthenogenesis, but merely dealt with it incidentally as a check upon his work on artificial parthenogenesis or chemical fertilization. He, therefore, merely points out the foregoing particulars without entering into any discussion as to their significance.

Spontaneous Membrane Formation.

In only one important point is there lack of essential agreement between his results and mine: he failed to note any cases of spontaneous membrane formation which was so frequently noted in my experiments. I am at a loss to explain this discrepancy between his results and mine, both performed at Pacific Grove and both unquestionably safeguarded against accidental error. Possibly the material behaves differently at different times of the year and Loeb's work was done at quite a different time from mine, which was confined to the last few days of April and the first three weeks of May. The only difference in treatment between Loeb's cultures and mine had to do with the methods of

sterilizing the sea water in order to avoid normal fertilization. Although he does not mention the fact in these particular experiments, his practice was to use boiled or highly heated sea water; while I used sea water that had been kept in a demijohn for at least four days. It seems barely possible that heating of the water prevents spontaneous membrane formation. I would, of course, have tried this experiment, had I known of Loeb's detailed paper at the time of my experiments, but I had with me only his book on "Artificial Parthenogenesis and Fertilization" and in that book he fails to mention the occurrence of spontaneous parthenogenesis in *Asterina*. Heated sea water would doubtless be relatively poor in oxygen and this might be responsible for his failure to find spontaneous membrane formation. It is possible also that this process (membrane formation) was so belated in its appearance that it occurred after Loeb had ceased to look for it in his cultures. Unless one gets a very early start in experiments with this material the working day is likely to be over before any signs of membrane formation appear, and the next morning these eggs will have undergone cytolysis and their membranes will have disappeared. My plan was to make a before-breakfast expedition to the collecting grounds, get the material ready for work, breakfast, and be ready for experimentation by 8:00 A.M. If one begins to collect after breakfast it is likely to be nearly 11:00 A.M. before experiments with *Asterina* eggs could be commenced. If such were the case it would be 6:00 P.M. before distinct membranes would be visible, later than the time when the investigator habitually "knocks off for the day." It is barely possible then that Loeb may have missed spontaneous membrane formation in some such way as this.

Of the validity of my obervations there seems to be no doubt. In answer to the criticism that failure to heat the sea-water vitiates the observations, it may be said that if these eggs with membranes are fertilized, they should segment; but they never do. We seem to be forced to the conclusion, therefore, that membrane formation in *Asterina,* which Loeb has been at such pains to bring about by chemical means, occurs spontaneously in a considerable percentage of cases. This being true, the various manipulations

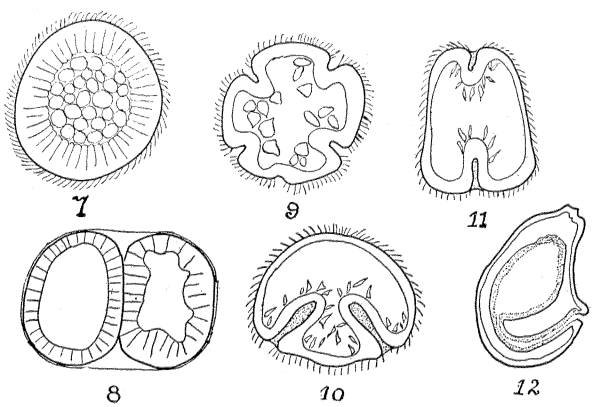
used by Loeb have merely served to hasten a natural process and to cause it to occur in a larger percentage of eggs.

Something in addition to membrane formation occurs in the eggs handled by Loeb, for they go ahead and segment as do normally fertilized eggs, while the eggs that form spontaneous membranes do not segment. From this it would appear that *the so-called "fertilization membrane" is not an essential feature of development, but merely its usual accompaniment.* Thus the voluminous literature dealing with artificial membrane formation, as though it were the most important event in the initiation of development, loses some of its force. Loeb himself recognized that development, at least in *Asterina,* could proceed without membrane formation; witness his statement, corroborated by my own observations, that spontaneous parthenogenesis proceeds without the preliminary of membrane formation. He seems to suggest, however, that this is not to be considered as typical development, since it begins later, goes more slowly and results less normally than in the case of fertilized eggs. Exactly similar results may be obtained, however, in fertilized eggs by the use of agents that retard development, such as cold, hybridization, anæsthetics, etc. So we must admit that *real development may occur without membrane formation, and that membrane formation may occur without initiation of development. The two processes are independent though they usually are associated in normal ontogeny.*

The Development Spontaneously of Parthenogenetic Eggs of Asterina.

According to Loeb, the development of the chemically fertilized eggs differs from that of the spontaneously fertilized eggs in two respects; first, in forming membranes; and second, in beginning earlier and proceeding more rapidly. With this distinction I fully agree. Evidently, in the chemically fertilized eggs, something in addition to membrane formation takes place, a something that results in prompt initiation of the changes expressed by cleavage. In the spontaneously parthenogenetic eggs, however, initiation to development is very slow in beginning, and

is less effective when it does begin. In last analysis the difference is evidently to be expressed in terms of rate of change. *The whole process of ontogeny in these eggs is from the beginning retarded, and the results are exactly similar to those which may be obtained by the use of growth-retarding agents applied to newly fertilized eggs.* The first effect of pronounced retardation of the normal growth process in the egg is the partial or complete obliteration of the characteristic axes of polarity and symmetry in the egg, a breaking down of the axial gradient. This results subsequently in loss of unity of organization, involving physiological isolation of blastomeres or of cell aggregates, in double and triple polarity and consequent double or triple monsters, and in a whole series of products of differential inhibition, such as those described by Child for sea-urchin.



FIGS. 7–12. Later developmental stages in spontaneously parthenogenetic eggs of *Asterina*. 7. The commonly occurring solid blastula type. 8. A pair of twin blastulæ enclosed within one vitelline membrane, evidently the result of physiological isolation of two blastomeres in the two-cell stage. 9. A multipolar embryo gastrulating at several points. 10. Double monster with two symmetrical archentera. 11. Another double monster with an additional anterior archenteron. 12. A microcephalic ciliated larva, with differentiated stomach and intestine, but no anterior parts.

Some of the types of inhibited larvæ found in cultures of spontaneously parthenogenetic *Asterina* eggs are shown and described in figures 7–12. The solid blastula is the commonest type, a type devoid of an axis of polarity (Fig. 7). Forms that are bipolar

and tripolar, etc., usually undergo gastrulation in two or more places (Figs. 9, 10, 11) and produce double and triple monsters, etc. The most nearly normal forms are decidedly abnormal early bipennaria larvæ in which the anterior parts are relatively inhibited. Such a form is shown in Fig. 12, in which the mouth never breaks through, oesophogus is not clearly differentiated, but stomach and intestine are well devevloped. Since I have in preparation a detailed paper on twinning in *Asterina,* in which I intend to discuss the physiology of twinning in general, I shall not enter further into an account of the various types of inhibited larvæ that result from spontaneous parthenogenesis, for the same types result from several other kinds of inhibiting factors. In closing I merely wish to emphasize this one point: that *the results of spontaneous parthenogenesis are those usually found to accompany early growth retardation.* For a summary of this paper the reader is referred to the eleven points referred to on pages 110–112 and to the italicized clauses in the general discussion.

I am greatly indebted to the Hopkins Marine Station of Leland Stanford University, and to its director, Dr. Walter K. Fisher, for the excellent facilities for research that I enjoyed while at Pacific Grove.

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